Interference Reflection Microscopy Shows Novel Insights to Bacterial Gliding Motility

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Introduction

The gliding motility of the Δ-proteobacterium, *Myxococcus xanthus* is used to facilitate either social or adventurous motility depending on the availability of nutrients in their environment. The size of bacteria limits our ability to use sectioning microscopy techniques, and so most studies on gliding motility use fluorescence-based techniques to focus on lateral (x, y) dynamics. **We aim to use interference reflection microscopy (IRM) to visualise the axial motility dynamics in gliding cells to better understand their underlying gliding motility mechanisms**.

- IRM provides increased axial information below the diffraction limit ($d_z \approx 90$nm)
- Interference fringe pattern is generated by interference of the reflected waves from the coverslip and specimen (Figure 1)
- IRM fringes are separated by a function of $\lambda/_{2n}$ and are $\lambda/_{4n}$ thick, where λ is the wavelength of incident light and n is the refractive index of the mounting medium

Methods

Strains & Culturing; *Myxococcus xanthus* (DK1622) was cultured in Double-Casitone Yeast Extract (DCYE) medium for approx. 48 hours at 30°C, 180rpm. Specimens were then mounted in DCYE medium (n = 1.34) prior to imaging. Fruiting bodies were cultured on Clone Fruiting (CF) medium at 30°C for 10 days.

Interference Reflection Microscopy; IRM was carried out using an FV1000 confocal laser scanning system coupled to an IX81 inverted microscope (Olympus, Japan) set up in reflection mode using a *60x/1.35NA U Plan S Apo Oil* objective lens (Olympus, Japan). Incident light was provided by a 488nm line from a GLG3135 Ar^+ laser (Showa Optronics, Japan) and the reflection signal was detected using a photomultiplier tube (Olympus, Japan).

Assessment of Gliding Motility; Gliding motility was assessed by applying a tracking algorithm (TrackMate[5]) to motile cells. Kymographs were used to determine changes in axial position of the gliding cells over time based on the interference fringe pattern.

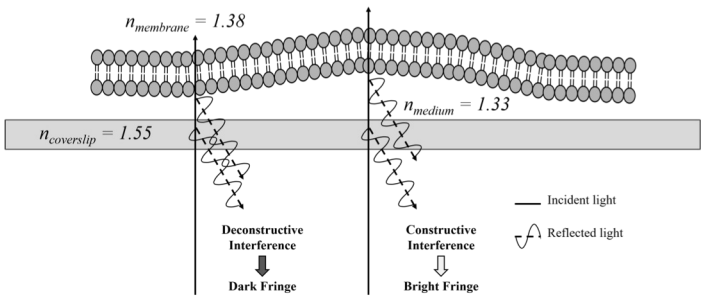
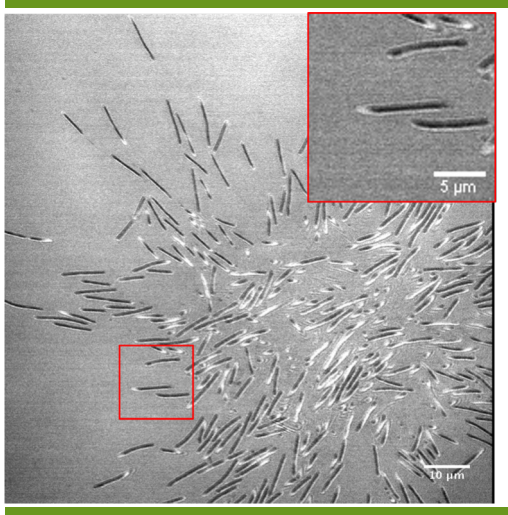


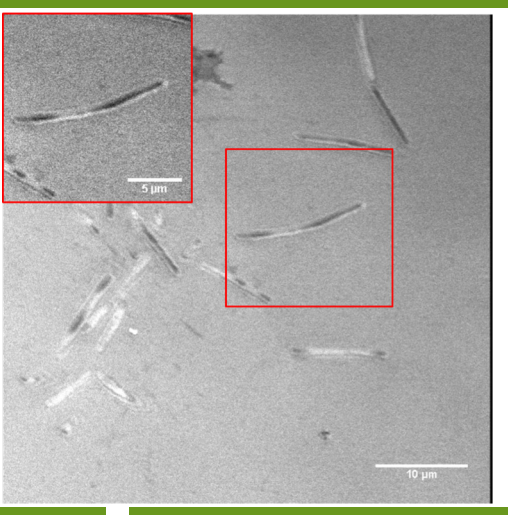
Figure 1. Principle of IRM. Incident light is reflected at refractive index boundaries. Reflected light waves from different boundaries interfere to generate a interference pattern which reveals axial information. The interference pattern forms dark and bright fringes corresponding to the height of the specimen from the coverslip.

Visualising Axial Changes in Gliding Cells



Motile cells periodically raise their leading pole during gliding

Figure 2. A single time-point showing *M. xanthus* cells gliding laterally across a coverslip captured using IRM. The insert shows a representative gliding cell which has an interference fringe present at the leading pole, indicating that this region is raised above the surface of the coverslip.



Gliding cells can exhibit an undulating motion which may be indicative of focal adhesion complexes

Figure 3. Gliding cells demonstrate a dampened wave-like motion during gliding motility. The interference fringe present in the centre of the cell shown indicates that the cell is adhered at the two poles, but raised in the centre. Over time this interference fringe migrates towards the lagging pole as the cell glides, resulting in a wave-like motion along the cell body.

Interpreting 3D Information provided by IRM

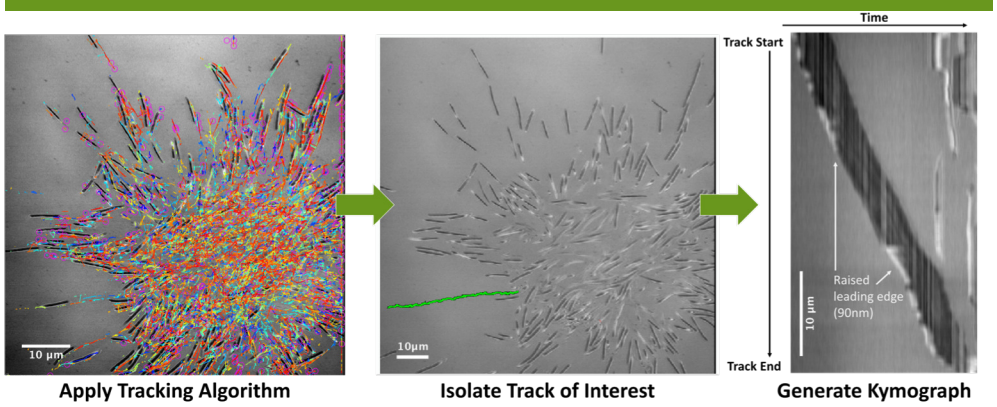
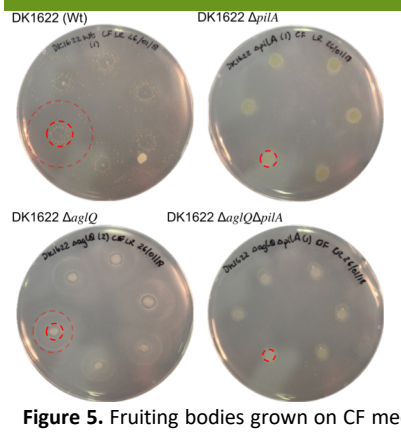


Figure 4. Gliding microcolony shown in Figure 2 has been analysed using TrackMate and a kymograph of a representative gliding cell is shown. The changes in intensity at the leading pole of the cell are the result of the cell being raised from the surface of the coverslip. Over the acquisition time (*ca.* 12 minutes) the cell periodically raises its leading pole during gliding via an unknown mechanism. Based on the interference pattern we can calculate that **the leading pole of the cell is raised 90nm above the coverslip** during gliding

Using Motility Mutants to Understand Gliding



Gliding motility relies on the Type IV Secretion System (T4SS) and cytoskeletal dynamics. **Using IRM to compare the axial movements of deletion mutants we can elucidate the mechanisms which underpin the changes in cell positioning during gliding.**

Figure 5. Fruiting bodies grown on CF medium. Differences in motility phenotypes can be observed between deletion mutants. Δ*pilA* lacks the major pilin subunit used in the T4SS and cannot attach and glide across surfaces. Δ*aglQ* are unable to use proton motive force (PMF) to rotate the MreB cytoskeleton and therefore do not glide as efficiently as the wide type (Wt). A double mutant (Δ*aglQ*Δ*pilA*) lacks the ability for both the T4SS and PMF and therefore is not motile.

Conclusions

- We present IRM as a label-free imaging technique which is able to determine changes in axial position of gliding bacteria below the diffraction limit
- We have shown that *M. xanthus* will periodically raise regions of the cell body above the underlying surface during gliding
- The mechanism which facilitates these changes remains unknown, however we hope to use motility mutants to investigate further

References

[1] Nan, *et al*., 2011. *PNAS*, **108**, 2498–2503; [2] Faure, *et al*., 2016. *Nature*, **539**, 530–535; [3] Zhang, *et al*., 2012. *FEMS Microbiology Reviews*, **36**, 149–164; [4] Tinevez, *et al*., 2016. *Methods*, **115**, 80-90

Acknowledgements

We would like to acknowledge David Whitworth (Aberystwyth University, Wales) for supplying the Wt DK1622 *M. xanthus* strain, and also to Lotte Søgaard-Andersen, Dobromir Szadkowski and Anke Treuner-Lange (MPI, Marburg) for ongoing discussion and supplying the motility deletion mutants.

RMS

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